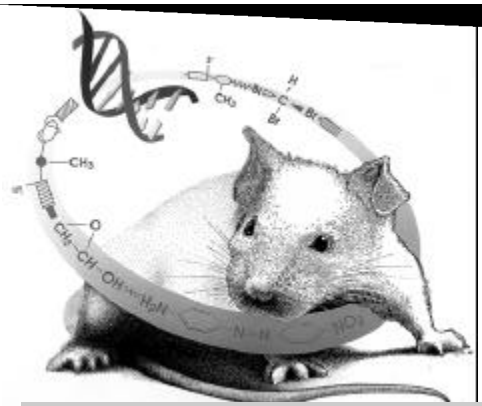

The Tg.AC Workgroup Newsletter

The Tg.AC Workgroup Newsletter is published by The Department of Toxicology and Safety Assessment, Boehringer Ingelheim Pharmaceuticals, Inc. as a means of communication for the HESI's Alternatives to Carcinogenicity Testing Committee.

Letter and article submissions are welcome. Persons interested in contributing to the newsletter should contact:

Sylvia M. Furst, Ph.D.
Boehringer Ingelheim Pharmaceuticals, Inc.
Research and Development Center
900 Ridgebury Rd.
P.O. Box 368
Ridgefield, CT 06877-0368

Tel: 203-791-6112
Fax: 203-789-5797
sfurst@rdg.boehringer-ingelheim.com

On the Inside:

Criteria for Evaluating Papilloma Responses in Tg.AC Mice: A Survey

Raymond Tice*, Thomas Goldsworthy*, Glenda Moser*, Joseph Clancy*, Jud Spalding**, Stanley Stasiewicz**, and Raymond Tennant**

*Integrated Laboratory Systems, and **NIEHS, Research Triangle Park, NC. *Supported by NIEHS Contract N01-ES-65399.

In the typical 26-week Tg.AC skin painting study, the endpoints of concern are the incidence and multiplicity of papillomas at the site of application. In initial Tg.AC validation tests conducted at ILS in support of NIEHS, the sites of individual papillomas were mapped as they appeared and followed weekly throughout the study. When it became apparent that the multiplicity of papillomas in responding Tg.AC mice could be quite high and that accurate quantitative mapping under these conditions was difficult and took appreciable resources, a decision was made to count but not to map individual papillomas. Based also on the high multiplicity of papillomas seen in responding mice treated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and on animal health concerns, a decision was made to not count papillomas on each mouse in excess of 30.

In evaluating papilloma data from Tg.AC studies, decision criteria have been used to identify, for each mouse: (i) the time between the onset of treatment and the first appearance of a papilloma, (ii) the maximum multiplicity of papillomas expressed during the treatment period; and (iii) the time to appearance of the maximum multiplicity of papillomas. In addition, criteria to exclude or include individual mice from analysis based on the length of time on study and the presence of papillomas were developed. Because of discussions about the appropriateness and utility of various criteria, we concluded that it would be useful to survey the Tg.AC scientific community for the decision criteria used to judge multiplicity and incidence. To aid in this survey, representative papilloma data are provided (see next page). The interested participant in this survey should provide a description of the criteria used to judge the suitability of papilloma data for subsequent analysis. Additionally, the maximum yield of papillomas, and the latency times for the first appearance of a papilloma, and the maximal appearance of papillomas should be provided by mouse identification number and by group. It is also important to present the statistical methods that would be used to analyze such data. This information should be faxed to Dr. R. Tice at 919-544-0380, or preferably sent by e-mail (rtice@ils-inc.com). The results of the survey, once tabulated, will be published in an upcoming Tg.AC newsletter.

INDIVIDUAL PAPILLOMA DATA

Individual Papilloma Yield (set 1) — **WEEKS OF TREATMENT**

Animal ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
030	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041	0	0	0	0	0	2	8	9	11	16	16	20	22	22	18	18	20	20	24	23	23	18	22	24	D	D
042	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
044	0	0	0	0	0	1	5	8	11	13	15	16	19	19	13	14	14	17	18	23	23	22	22	26	26	25
049	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
051	0	0	0	0	0	0	1	7	10	15	15	16	16	18	14	14	14	16	18	18	20	18	19	14	15	15
073	0	0	0	0	0	0	0	0	1	3	3	3	8	10	18	20	20	20	22	27	23	21	18	15	13	13
076	0	0	0	1	1	0	0	0	1	1	2	3	4	2	1	0	0	3	3	0	0	2	2	0	0	0
088	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
090	0	0	0	0	0	0	2	4	10	10	13	12	13	13	9	9	11	11	12	10	10	15	12	7	4	3

Individual Papilloma Yield (set 2) — **WEEKS OF TREATMENT**

Animal ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
104	0	0	0	1	2	6	6	7	8	18	19	16	19	18	20	24	26	28	22	20	21	22	23	29	28	13
111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	6	11
128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	4
132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	2
135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	3
136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0
146	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	6	14
157	0	0	0	0	0	0	0	0	2	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
161	0	0	0	0	0	1	1	3	5	3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Individual Papilloma Yield (set 3) — **WEEKS OF TREATMENT**

Animal ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
092	0	0	0	0	1	1	2	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
097	0	0	0	0	1	1	2	3	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	4	10	11	21	27	27	27	28	30	28	17	24	25	30	27	29	25	25	18	19	19
109	0	0	0	0	0	0	2	2	6	7	8	9	13	12	13	10	10	10	13	15	15	16	16	17	15	16
110	0	0	0	0	0	0	2	3	5	8	8	11	13	11	13	D	D	D	D	D	D	D	D	D	D	D
125	0	0	0	0	0	0	0	4	5	0	13	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
133	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
143	0	0	0	0	0	0	0	0	0	1	1	1	1	8	8	9	9	12	10	11	12	D	D	D	D	D
156	0	0	0	0	0	0	5	8	10	13	12	12	12	12	15	15	12	15	15	D	D	D	D	D	D	D
164	0	0	0	0	0	0	0	3	4	5	5	7	11	11	13	13	14	14	17	18	18	18	18	19	18	19

D = dead

An Update On Studies Being Conducted By The National Toxicology Program

by Skip Eastin, NTP

The NTP is currently evaluating the response in both sexes of homozygous Tg.AC mice to three pairs of chemicals from the ILSI alternative models list. In addition to determining the carcinogenic potential, we are further evaluating the utility of this model by comparing the tumor response of the traditional topical application with that of an oral exposure. We are evaluating two peroxisome proliferators (WY-14643 and di(2-ethyhexyl) phthalate), two estrogenic drugs (ethinylestradiol and diethylstilbestrol), and two antineoplastic drugs (cyclophosphamide and melphalan).

Range-finding studies for WY-14643 and di(2-ethyhexyl) phthalate (DEHP) were not conducted because these two chemicals had recently undergone evaluation in the NTP bioassay and doses for the Tg.AC mice were set based on the toxicity results from the studies with B6C3F1 mice. The doses used for DEHP 26-week studies were 0, 100, 200 and 400 mg/kg in acetone in the topical studies and 0, 1500, 3000, 6000 ppm in the dosed-feed studies. The doses used for the WY-14643 26-week studies were 0, 2, 10 and 20 mg/kg in acetone for the topical studies and 0, 10, 50 and 100 ppm in the dosed-feed studies. In addition to the standard protocol requirements, peroxisomal enzyme activity (b-oxidation, acyl CoA oxidase) and cell proliferation (PCNA, CDK) were determined in liver. These studies were initiated in February 1997 and TPA controls were not added as routine at this time. **Both studies have been completed and the data are in evaluation by NTP**.

Cyclophosphamide and melphalan range-finding studies were completed. Doses for the 26-week cyclophosphamide topical studies were set at 0, 10, 30, or 90 mg/kg for Tg.AC and 0 and 90 for FVB mice 2x/wk; separate groups of Tg.AC mice received 1.25 µg TPA/animal 3x/wk. The oral gavage cyclophosphamide study doses were set at 0, 10, 30, and 60 mg/kg in corn oil in Tg.AC and 0 and 60 mg/kg in FVB mice. The doses were based on the results of the range-finding studies and on the results of Dr. Gerald Long's 26-week gavage studies in $p53^{+/-}$ mice. **Both topical and gavage cyclophosphamide 26-week studies started in November 1998**. Based on the results of the melphalan topical studies, doses for the 26-week studies were set at 0, 0.25, 1.0, and 4.0 mg/kg in methanol 1x/wk. The melphalan gavage range-finding studies had mortalities due to difficulties in chemical administration that precluded dose selection. A second set of range-finding studies were conducted using FVB mice. In these studies the survival was excellent. Results from this second range-finding study were used to set doses for the 26-week studies at 0, 0.25, 1.0, and 4.0 mg/kg in corn oil 1x/wk and 0 and 4.0 for FVB mice 1x/wk; separate groups of Tg.AC mice will receive 1.25 µg TPA/animal 3x/wk. **The melphalan 26-week studies started in May 1999**.

The results of the estrogenic drug range-finding studies with Tg.AC mice were used to set dose levels for the 26-week studies. The doses for ethinylestradiol for both routes of exposure were set at 0, 33, 265, 530 µg/kg 2x/wk in 1:1 ethanol:water for the topical studies and in 0.7% methylcellulose for the gavage studies. Dose levels equimolar to those of the ethinylestradiol studies were set for the DES studies at 0, 30, 240, and 480 µg/kg 2x/wk in 95% ethanol for the topical studies and corn oil for the gavage studies. All studies have FVB mice receiving control and high doses of drug and topical studies also have separate groups of Tg.AC mice receiving 1.25 µg TPA/animal 3x/wk. **These estrogenic drug 26-week studies started in July 1999.**

NOTE: all of the aforementioned studies are using homozygous Tg.AC mice

Contact: Dr. Skip Eastin (919) 541-7941
eastin@niehs.nih.gov

Vehicle Comparison Study in the Hemizygous Tg.AC Mouse

by S.M. Furst, BIPI

The Department of Toxicology and Safety Assessment at Boehringer Ingelheim Pharmaceuticals, Inc. in Ridgefield, CT is currently investigating the potential effects of various vehicles on the response of hemizygous Tg.AC mice to dermal administration of phorbol 12-myristate 13-acetate (PMA). The study was initiated on July 19, 1999 and will run for 26 weeks until termination on January 17, 2000. Twelve groups of 15 animals/sex are being given acetone as a control and PMA three times weekly in various vehicles dermally. Vehicles include acetone, DMSO, 100% ethanol, 70% ethanol, 100% methanol, acetone/DMSO (4:1), acetone/DMSO (1:1), acetone/ethanol (1:1), acetone/olive oil (4:1). The PMA is administered at 2.5 ug/mouse for all dose groups except the acetone control. Two additional groups are receiving PMA at a dose level of 1.25 ug/mouse in DMSO and in 100% ethanol. Mice are observed daily for morbidity/mortality and clinical signs of toxicity. Animals are also examined weekly for external skin masses or subcutaneous masses. Body weights and food consumption for each animal is measured weekly.

A B C D

At the end of the 26 week dosing period a complete necropsy with collection of approximately 50 tissues and histopathological examination of approximately 20 tissues will be performed. The final report is scheduled to be issued in June, 2000.

Contact : S.M. Furst at (203) 791-6112
sfurst@rdg.boehringer-ingelheim.com

Articles of Interest

Delker D, Yano BL and Gollapudi B. (1999) v-Ha-ras gene expression in liver and kidney of transgenic Tg.AC mice following chemically induced tissue injury. Toxicological Sciences 50: 90-97.

Humble MC, Szczesniak CJ, Luetteke NC, Spalding JW, Cannon RE, Hansen LA, Lee DC and Tennant RW. (1998) TGF alpha is dispensable for skin tumorigenesis in Tg.AC mice. Toxicologic Pathology Jul-Aug; 26(4):562-569.

Cannon RE, Spalding JW, Trempus CS, Szczesniak CJ, Virgil KM, Humble MC, Tennant RW. (1997) Kinetics of wound-induced v-Ha-ras transgene expression and papilloma development in transgenic Tg.AC mice. Molecular Carcinogenesis 20(1):108-114.

Updated List of Chemicals Tested in Tg·AC Mice

Compiled by J.W. Spalding, S. Stasiewicz, and R.W. Tennant, NIEHS, RTP, NC

A summary of results for 52 chemicals tested in Tg·AC mice is listed below. Literature references and genotypes (homozygous or hemizygous) are indicated as well as the results of chemical exposure in the NTP 2-year conventional rodent bioassay.

Chemical	Route [1]	Genotoxicity	Bioassay Results [2]	Results in Tg·AC
1-Chloro-2-methyl propene [3]	Gavage	+ [5]	+	+ [8]
2,4-Diaminotoluene [4]		+	+	+ [10]
7,12-Dimethylbenzanthracene [3]		+	+	+ [11]
Benzene [3,4]	Skin Paint [2,3] Gavage ( ) [3]	+ [5]	+	+ [12,13]
Melphalan [4]		+	+ [9]	+ [10]
Methyl ethyl ketone peroxide [3]		+	+	+ [11]
p-Cresidine [3]		+	+	+ [14]
Urethane [3]	I.P.	+ [5]	+	+ [11]
Ethyl acrylate [3]		+	+	[15]
Glycidol [3]	Skin Paint Gavage	+	+	[8]
2,4-dinitro-1-fluorobenzene [2]		+	**nt**	+ [20]
Diisopropylcarbodiimide [4]		+ [5]	**nt**	[16]
1-Chloro-2-propanol [4]		+		[17]
2-Chloroethanol [3]		+		[12]
2,6-Diaminotoluene [4]		+		[10]
8-Hydroxyquinoline [4]		+		[10]
p-Anisidine [4]		+		[10]
Tripropylene glycol diacrylate [3]		**nt** [7]	**nt**	+ [15]
Fluocinolone acetonide [2]		**nt** [7]	**nt**	[20]
2,3,7,8-Tetrachlorodibenzodioxin [3]			+	+ [10]
Benzoyl peroxide [3,4]			+	+ [11]

Cyclosporin [4]	Gavage		+ [7]	+ [10]
Diethylstilbestrol [4]			+ [7]	+ [10]
Lauric acid diethanolamine [3,4]			+	+ [17]
Mirex [3]			+	+ [14]
o-Benzyl-p-chlorophenol [3]			+	+ [12]
Oxymetholone [4]			+	+ [18]
Pentachlorophenol [4]			+	+ [17]
TPA [3,4]			+	+ [11,12]
Chloroform	Gavage		+	[19]
Chloroprene [3]	Inhalation		+	[8]
Coconut oil diethanolamine [4]			+	[17]
Di-(2-ethylhexyl) phthalate [3]	Skin Paint Gavage		+	[8]
Diethanolamine [3]			+	[17]
Furfuryl alcohol [4]			+	[17]
Methylphenidate [4]			+	[8]
N-Methylolacrylamide [4]			+	[10]
Pyridine [4]			+	[17]
Triethanolamine [3]			+	[12]
Wy-14643 [3]	Skin Paint Feed		+	[8]
Dicyclohexylcarbodiimide [4]			**nt**	+ [16]
Mezerein [3]			**nt**	+ [8]
Sodium arsenite [2]	Water		**nt**	+ [21]
Acetic acid [3]			**nt**	[11]
Acetone [3,4]			**nt**	[11,12]
Resorcinol [4]				+ [10]
Rotenone [4]				+ [10]
70% or 95% Ethanol [3,4]				[12]

Benzethonium chloride [3]				12
Oleic acid diethanolamine [4]				17
Phenol [3]				

1 Route of administration was by skin paint unless otherwise indicated.
2 The results are from the NTP two-year rodent bioassay unless indicated.
3 Homozygous Tg·AC mice
4 Hemizygous Tg·AC mice
5 Micronucleus positive
6 On Test
7 Not Tested in the NTP two-year rodent bioassay
8 Unpublished data (Laboratory of Environmental Carcinogenesis and Mutagenesis or National Toxicology Program/NIEHS)

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21 Sodium arsenite acted as an enhancer of papillogenesis; Germolec, D., Spalding, J., Boorman, G., Wilmer, J., Yoshida, T., Simeonova, P., Bruccoleri, A., Kayama, F., Gaido, K., Tennant, R., Burleson, F., Dong, W., Lang, R. and Luster, M. (1997) Arsenic can mediate skin neoplasia by chronic stimulation of keratinocyte-derived growth factors, *Mutation Research* **386**, 209-218; Germolec, D.R., Spalding, J., Yu, H.-S., Chen, G.S., Simeonova, P.P., Humble, M.C., Bruccoleri, A., Boorman, G.A., Foley, J.F., Yoshida, T. and Luster, M.I. (1998) Arsenic enhancement of skin neoplasia by chronic stimulation of growth factors, *American Journal of Pathology* **153**, 1775-1785.

Websites of Interest

TRANSGENIC PUBLICATIONS

http://www-mp.ucdavis.edu/tgmice/Mambio/TGREFABS.htm

This website provides a list of publications regarding transgenic mice and centers on the pathology of various tumors. The site is based on publications provided by the UC Davis Transgenic Pathology Facility. Abstracts and illustrations are available including photomicrographs of various tumors and audiovisual presentations.

THE CARCINOGENIC POTENCY PROJECT

http://potency.berkeley.edu/cpdb/html

The carcinogenic potency project: This site provides a carcinogenic potency database including information and results of chronic, long-term animal cancer tests such as those conducted by the NCI/NTP. Analyses of 5152 experiments on 1298 chemicals are provided.

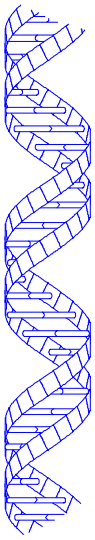
Upcoming Meetings and Events

☺ 3rd World Congress on Alternatives and Animal Use in the Life Sciences
Bologna, Italy

August 29– September 2, 1999

☺ 9th North American ISSX Meeting
Opryland Hotel, Nashville, TN

October 24-28, 1999



A B C D

S. Furst, RD 9-5
Research & Development Center
900 Ridgebury Rd./PO Box 368
Ridgebury, CT 06877-0368

The Tg.AC Workgroup Newsletter